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# Development and Deployment of a Bioreactor for the Removal of Sulfate and Manganese from Circumneutral Coal Mine Drainage

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*Chapter 4*

**DEVELOPMENT AND DEPLOYMENT  
OF A BIOREACTOR FOR THE REMOVAL OF SULFATE  
AND MANGANESE FROM CIRCUMNEUTRAL COAL  
MINE DRAINAGE**

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**Abstract**

Surface mining, in the form of contour mining and mountain-top removal, is a common means for retrieving coal in the Appalachian Coal Belt region of Kentucky. Overburden or excess spoil generated by these two methods is placed in valley fills. Traditionally Constructed fills have been shown to adversely impact headwater ecosystems via stream burial and through alterations to the hydrology, sediment supply, water quality and biological composition of downstream reaches. Mine drainages emanating from the toe of valley fills often contain elevated levels of total dissolved solids and heavy metals. Drainage chemistry from Guy Cove, a valley fill located in eastern Kentucky, exhibited a mean pH of 6.5 and Fe, Mn and SO<sub>4</sub> concentrations of 1.5, 14, and 1264 mg L<sup>-1</sup>, respectively. The objective of this research was to develop an anaerobic bioreactor for the purpose of reducing Mn and SO<sub>4</sub> concentrations in the mine drainage. Development began with batch experiments that tested five different organic carbon sources and five different inorganic substrates. A synthetic mine drainage with a pH of 6.2 and Mn and SO<sub>4</sub> concentrations of 90 and 1,500 mg/L, respectively, was used in the experiment. Manganese and SO<sub>4</sub> removal varied widely between treatment matrices, with removal rates <10 to 100% for Mn and <10 to >80% for SO<sub>4</sub>. The substrate sources which provided the most treatment were hardwood mulch and biosolids combined with creek sediment. Subsequent experiments were performed using the synthetic mine

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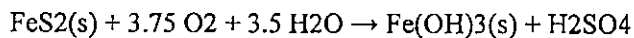
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drainage in small bioreactors (55 liter plastic tanks) filled with creek sediment with either hardwood mulch or biosolids, each replicated three times. Over a 65 day treatment period >90% of the Mn and 70% of the SO<sub>4</sub> was removed. There were no statistical differences between the two organic substrates. Using this information, in-situ bioreactors consisting of two 5,500 liter plastic septic tanks filled with creek sediment, hardwood mulch and manure compost were installed at Guy Cove. Mine drainage was collected in a sump and conveyed through the inline bioreactors by gravity. Gate valves were used to control flow through the bioreactors. After a 10-month monitoring period, the in-situ bioreactors removed 12, 11, and 64% of Mn, SO<sub>4</sub> and Fe from the drainage, respectively. Results from the field differed greatly from those observed under controlled laboratory conditions. Efforts to improve the efficiency of the in-situ bioreactors are underway.

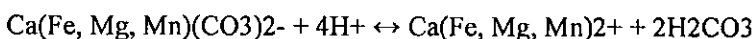
## Introduction

The Appalachian region of the U.S. is a land of contrast - economic hardships are prevalent, but the region abounds with natural resources. Appalachian forests support some of the greatest biological diversity in the world's temperate region, but extraction of the region's abundant coal reserves has impacted the landscape. Since 1977, over 600,000 hectares of Appalachian forest have been affected by surface mining, producing significant economic, environmental, and ecological challenges. Surface mining practices in Appalachia, requiring valley fills, have resulted in the burial of more than 1,150 kilometers of perennial streams by more than 6,800 valley fills (USEPA 2005) and some estimates suggest that over 3,200 kilometers of streams are buried (Schoof 2010). Impacts from these practices have not only resulted in stream and habitat loss, but many studies also show that this mining method affects downstream water quality and aquatic communities (Palmer et al. 2010, Pond 2004, Winterbourn 2000, and Anthony 1999). Although great strides were made for protecting water resources from surface mining with the passage of the U.S. federal Surface Mining Control and Reclamation Act (SMCRA) in 1977, water quality issues remain.

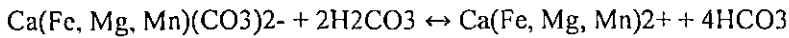
Surface mining can severely decrease the quality of water discharging from a watershed (Fritz 2010, Pond 2004). Mine drainage is often enriched with elevated levels of total dissolved solids, created through the oxidation of pyrite (FeS<sub>2</sub>) and weathering of other geologic minerals found in the coal seam and associated strata. When pyrite is exposed to water and air, it oxidizes and forms an insoluble iron hydroxide and sulfuric acid following the reaction:



There are many factors that contribute to the formation and intensity of mine drainage. These factors include pH, mineral surface area, oxygen concentration, and bacterial activity (Evangelou 1999). Mining practices break-up geological strata and increase the surface area of extracted rock. With this increased surface area, more reaction sites are available for oxidation to occur (Nodrostrom and Alpers 1999). The oxidation of pyrite is an acid producing reaction, but when influenced by certain geological strata, drainage can be buffered to near neutral or alkaline, due to the dissolution of carbonates by sulfuric acid. For instance, ankerite, a common carbonate found in eastern Kentucky coal mines (Wunsch et al. 1996), buffers the acidity produced through the oxidation of pyrite by the following chemical equations.



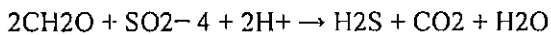
-produces carbonic acid



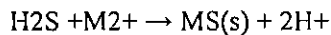
-produces bicarbonate alkalinity

Although this net increase in alkalinity and pH allows for the precipitation of some metals from solution, Mn remains problematic due to its high solubility at circumneutral pHs (Edwards 2008). Moreover, elevated concentrations of dissolved ions are often observed in mine drainages, even with high pHs.

Strong linear relationships have been shown between elevated concentrations of dissolved ions, particularly sulfate, and decreased stream biodiversity (Palmer et al. 2009). Use of a passive treatment system and sulfate reducing bacteria (SRB) could be used to remove metals and sulfate from mine drainage via sulfate reduction and improve stream quality. The process by which SRB mitigate mine drainage involves a series of chemical reactions where sulfate is reduced to sulfide following the equation:



where  $\text{CH}_2\text{O}$  represents an organic substrate. Subsequently, sulfide bonds with metals present in the mine drainage to form an insoluble metal sulfide precipitate following the equation:



where M represents a metal such as Fe, Zn, Ni and Pb (Gibert, et al. 2002). Formation of sulfide-metal compounds is a good way to mitigate drainage due to the fact that these compounds are highly insoluble (Wildeman and Updegraff 1997).

SRB are only effective in environments conducive to their growth and production. Limiting factors to the effectiveness of SRBs are pH, oxidation reduction potential (Eh), organic carbon and sulfate. Numerous studies have shown the effects of pH on SRB, and concluded that a pH between 5.5 and 8 is ideal for the production of SRB (Gibert et al. 2002, Hao et al. 1996). However, there have also been successful studies showing that SRB can function at a pH below 3 (Neculita et al. 2007). The Eh level that SRB prefer have generally been found to be no higher than -100 mV (Gibert et al. 2002, Postgate 1984), but SRB have been found in sites with positive Eh values (Neculita et al. 2007). With the pH and Eh being somewhat variable, organic carbon and sulfate sources become vital components. In coal mine drainage, sulfate is readily available through the oxidation of pyrite, but organic carbon is generally not available and must be introduced.

Numerous sources of organic carbon (woodchips or dust, livestock manure, or hay) can be used for sulfate reduction. Even though SRB are generally present in many natural systems (Hao et al. 1996), a mixture of different organic carbon sources has been found to provide the best substrate for sulfate reduction. An easily accessible source is generally considered the preferred material due to lower transportation costs (Neculita et al. 2007, Wybrant et al. 1998).

SRB have successfully been tested in the abatement of mine drainage (Benner et al. 2002, Wybrant et al. 1998, Christensen et al. 1996). Using SRB, Benner et al. (1997 and 1999) observed up to 74% and 70% reduction in sulfate. Doshi (2006) concluded in a review of sulfate reducing bacteria studies using both bioreactors and permeable reactive barriers that SRBs were successful at reducing sulfate by nearly 100%. Waybrant et al. (1998) attained

greater than 99% sulfate removal, while Champagne (2005) showed 73% removal of sulfates. Chockalingam and Subramanian (2006) compared the differences between real mine drainage and synthetic drainage and found sulfate removal rates of 40% and 73%, respectively. Given these results, a study was initiated to develop and test a sulfate reducing bioreactor for treatment of sulfate and Mn enriched mine drainage from a surface mine in eastern Kentucky. The study involved three phases: 1) laboratory bench experiment with synthetic mine drainage to determine a suitable SRB substrate for treating Mn and  $\text{SO}_4$  enriched water, 2) larger (55 liter tanks) mesocosm experiment with synthetic mine drainage to further test SRB substrate suitability over a long duration (65 days), and 3) field deployment with circumneutral mine drainage on a surface mine using large (5,500 liter tanks) bioreactors.

## Phase 1: Laboratory Examination of SRB Substrates

The first phase of this experiment tested several different combinations of inorganic and organic substrates for the removal of manganese by sulfate reduction from a synthetic mine drainage, using laboratory bench-scale bioreactors. A detailed overview of the experiment was described by Karathanasis et al. (2010).

## Materials and Methods

Five inorganic substrates and five organic amendments were chosen by their availability and cost. The inorganic substrates used in the study were limestone, marble, creek sediment, polished river gravel, and sand. The organic substrates used in the study were corn mash, soybean oil, wood mulch, sorghum syrup, and biosolids. Combining the substrates and the amendments created 25 different duplicated treatments for use in the batch experiments. The following combinations were tested: creek sediment and biosolids, creek sediment and corn mash, creek sediment and mulch, creek sediment and soybean oil, creek sediment and sorghum syrup, and creek sediment without an amendment. A control treatment, consisting of no substrate or amendment, was also used. The synthetic mine drainage had a pH of 6.2 and contained approximately  $90 \text{ mg L}^{-1}$  Mn and  $1,500 \text{ mg L}^{-1}$   $\text{SO}_4^{2-}$ . Reagent grade manganese sulfate ( $\text{MnSO}_4$ ) and calcium sulfate dihydrate ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) were used as the  $\text{Mn}^{2+}$  and  $\text{SO}_4^{2-}$  sources (Fisher Scientific). The mixtures were tested in batch experiments using acid-washed glass 1-L suction flasks. The substrates and amendments were mixed in each flask on a 10:1 mass to mass ratio. The amendments were added on an oven-dried mass basis, except for the oils. The density ratio of the oils was used to determine the requisite masses of each. The mine drainage was added on an equivalent (1:1) mass/mass or mass/volume ratio to the substrate. In order to enforce and maintain an anaerobic environment in the flasks, purified nitrogen gas was flushed through the system continuously at a rate of  $0.014 \text{ m}^3 \text{ h}^{-1}$ . A gas trap was installed on each flask and filled with sodium thiosulfate to allow the nitrogen gas to leave the flask without allowing oxygen to enter the system.

## Sample Collection and Analysis

A sample was extracted from the flask daily for 3 weeks (21 days) or until the major constituents reached a stable concentration, and was analyzed for Eh, pH, Mn and  $\text{SO}_4$ . The Eh and pH were measured using a Fisher Scientific Accumet AP62 pH/mV meter

immediately after sample collection, using an Accumet pH probe and a silver/silver chloride Mettler Toledo InLab redox probe, with values adjusted to reflect a hydrogen reference electrode. A subsample pipetted from each flask was filtered through a 0.45  $\mu\text{m}$  membrane filter and preserved in 1-N hydrochloric acid for Mn and  $\text{SO}_4$  analysis. Manganese levels were tested using a Solaar M5 Thermo-Elemental atomic absorption spectrometer. Sulfate concentrations were analyzed by a variation of APHA Method 4,500- $\text{SO}_4^{2-}$  E (APHA 1998), using a Molecular Devices Versamay tunable plate reader at 450 nm (D'Angelo et al. 2001).

## Results

Manganese removal rates ranged from 100% to <10% for the combinations tested and sulfate removal rates were <10 to >80%. Removal rates were greatest in combinations containing either the biosolids or the wood mulch amendments. Due to the carbonate chemistry of limestone, rhodochrosite formation was feasible from the dissolution elements, and the sand treatments allowed bacteria to form microclimates suitable for their needs. The creek sediment encompassed both of these aspects, as well as native microbial communities suitable for large organic molecule decomposition and sulfate reduction. Thermodynamic data indicated that no single removal mechanism (sorption, sulfide, oxide, and/or carbonate formation) was capable of removing all the Mn from solution in these treatments. Consequently, at least two, and most likely all, of the removal mechanisms were effectively working in most treatments to varying degrees and similar results were expected for the second phase of the research.

## Phase 2: Small Scale Bioreactor

Based upon the results of the batch experiments, the most favorable choice for the second phase of the experiment was the creek sediment and biosolid treatment combination. However, due to the volume of biosolids required and the density of the material, which would inhibit the percolation rate of the mine drainage solution, a combination of the wood mulch and the biosolid amendments were used in the small scale bioreactor. A detailed overview of the experiment was described by Edwards et al. (2009).

## Materials and Methods

The same synthetic mine drainage used in the previous phase was used in the small scale bioreactors. The creek sediment was collected from a small undisturbed stream at the University of Kentucky's Robinson Forest in eastern Kentucky. The mulch used in the bioreactors was collected from a University of Kentucky research farm and is composed primarily of woody debris. The biosolids were collected from the Winchester, Kentucky wastewater treatment plant.

This study was conducted in a greenhouse and not exposed to temperature variations or weathering conditions. The temperature was approximately 25° C for the duration of the experiment. The bioreactors used in the study were 55 liter plastic tanks obtained from US Plastics Corp. (Lima, OH, USA) and were manufactured by Ace Roto-Mold. Each tank was plumbed such that it would be an upflow treatment system. The plumbing used in the system

was one-half inch diameter (1.3 cm) PVC pipe and all joints were sealed with thread tape, PVC cement, and/or caulk, as necessary. Synthetic mine drainage was pumped into the bioreactors using a peristaltic pump. The solution was pumped into a stand pipe that was connected to a perforated pipe that lay on the bottom of the tank. The solution then flowed upward through the SRB substrate material and drained out through another piece of PVC located near the top of the tank opposite the end where the standpipe was located. The outlet and perforated pipes were both wrapped in cheesecloth to prevent clogging. A gas trap filled with a saturated sodium thiosulfate solution was attached to the system to allow gases to escape and prevent oxygen from entering the system.

The treatment material was mixed in a 10:1 creek sediment to organic amendment ratio on a mass basis. Approximately two-thirds of each tank was filled with the substrate and amendment mixture. Three tanks were used to replicate one treatment, consisting of only wood mulch as the amendment. Three other tanks were used to replicate the second treatment with a combination of the wood mulch and approximately 15% biosolids. Synthetic drainage was pumped at an approximate rate of 1 mL per minute. A redox probe was installed in each tank to provide internal Eh measurements.

### Sample Collection and Analysis

An effluent sample from each tank was collected every day and analyzed for Mn,  $\text{SO}_4$ , pH, Eh and electrical conductivity (EC). In addition, the redox status within each tank was measured daily and the total amount of effluent produced was recorded. EC was measured using a Hanna Combo pH/EC meter. The pH and Eh were measured with a Fisher Scientific Accumet AP62 pH/mV meter using an Accumet pH probe and a silver/silver chloride Mettler Toledo InLab Redox Probe, respectively. The Eh values were corrected to reflect a standard hydrogen electrode. Manganese concentrations were tested using a Solaar M5 ThermoElemental Atomic Absorption Spectrometer. Sulfate concentrations were analyzed by a variation of APHA Method 4500- $\text{SO}_4^{2-}$  E (APHA, 1998). The method has been adapted for use on a Molecular Devices Versamay Tunable Plate Reader, analyzed at 450 nm.

### Results

The bioreactors successfully achieved greater than 90% reduction of the Mn from solution and more than 70% reduction of the  $\text{SO}_4$  from solution over the 65-day. No significant differences were observed between the two treatments. However, the treatment with the addition of biosolids removed 93.1% Mn, whereas the mulch treatment removed 92.5%. Both treatments achieved greater than 70%  $\text{SO}_4$  removal from the synthetic drainage. Sulfate reducing conditions were obtained in all tanks. The redox levels were below 200 mV. The treatments achieved a mean pH of 7.5 and mean Eh of -250mV making the formation of a  $\text{MnS}$  precipitate possible. A sequential extraction analysis showed, as hypothesized, a multitude of removal mechanisms (residual, crystalline, poorly crystalline, organically bound, exchangeable, water soluble) with the majority of the Mn sorbed to the organically bound phase and similar results were expected from phase three.



## Phase 3: Field Deployment

Using information generated from the previous studies, a larger scaled experiment was initiated at a surface mine site in eastern Kentucky that was discharging  $\text{SO}_4$  and Mn enriched circumneutral mine drainage.

### Materials and Methods

#### Study Site

The study was initiated at the University of Kentucky's Robinson Forest located in eastern Kentucky. The forest occupies 6,000 hectares across Breathitt, Perry, and Knott counties (Figure 1). A contiguous block containing over 4,000 hectares comprises the majority of the forest making it one of the largest of its kind in the region. The remainder of the forest is comprised of several outlying tracts that include the Laurel Fork tract which is nearly 1,000 hectares in size. Robinson Forest lies in the Cumberland Plateau physiographic region and is underlain by sandstones, shales, and coals (Cranfill 1980).

The main block of the forest contains a second generation mixed mesophytic forest approximately 90 years in age. In the early 1990's, the University leased the outlying tracts for mining. Laurel Fork was one of those tracts. Laurel Fork was reclaimed as forest land with areas of open pasture. Reclamation of the area included planting numerous pine species and mixed hardwoods. Located within Laurel Fork is a tributary named Guy Cove (Figure 1). Guy Cove is an approximately 41 hectare headwater catchment that was surface mined in the late 1990s. Approximately 1.8 million  $\text{m}^3$  of spoil was placed in the watershed's valley creating a typical head of hollow fill. The fill is drained by an underdrain and two surface rock drains. Water quality of Guy Cove is poor with elevated levels of  $\text{SO}_4$ , Mn, and Fe, which is typical of mine drainage from this area (Fritz et al. 2010). In an effort to improve water quality and habitat, a stream restoration project was initiated at Guy Cove in 2008 (Agouridis et al. 2009) using natural channel design procedures (Rosgen 2006). The stream creation required for approximately 114,700  $\text{m}^3$  of fill to be excavated and relocated to create a more naturally shaped valley and provide a suitable substrate for reestablishing a riparian forest.

The constructed stream begins in the head of the watershed and flows along the left side (facing downstream) of the valley fill, the gradient in the top portion of the constructed stream is moderate creating a meandering pool riffle stream configuration. As flow continues downstream, the gradient increases and sinuosity of the stream decreases and forms a step pool configuration. Numerous in-stream structures were used such as log steps and log/rock cross vanes to control gradient through-out the stream. As the stream reaches the crest of the valley fill, the stream flows through the modified left rock drain. Reaching the toe of the fill, the stream becomes a fairly straight slightly sloping channel flowing along the left side (facing downstream) (Figure 2). Mine seepage from the underdrain was collected in a pond on the right side of the watershed and flowed, by gravity, through the bioreactor and passive treatment system.

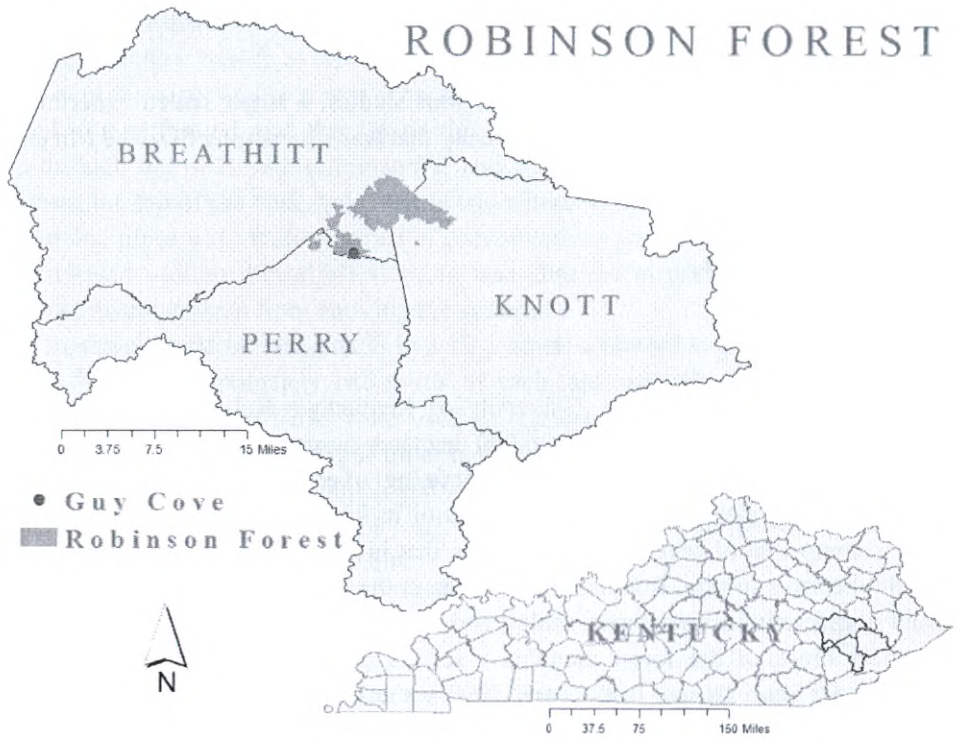


Figure 1. Locations of Robinson Forest and Guy Cove.

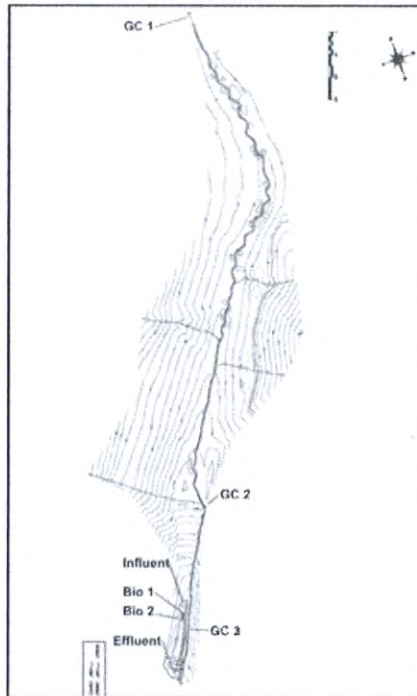


Figure 2. Schematic of the Guy Cove sampling points within the watershed. Modified from Agouridis et al. 2010.

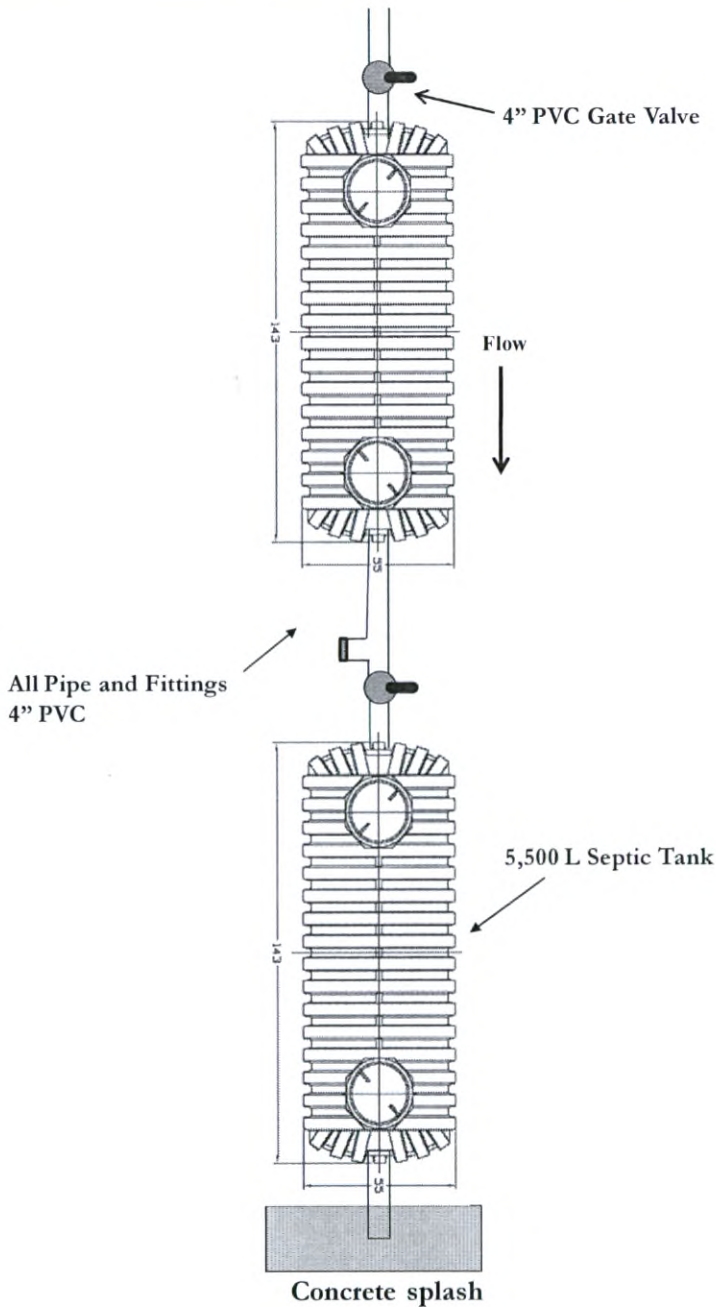


Figure 3. Bioreactor layout.

### Bioreactor Design

The bioreactors were constructed from two 5,500 liter Norwesco Bruiser Tanks - 1 Compartment / 2 Manholes (Bio 1); 2 Compartment / 2 Manholes (Bio 2) PVC septic tanks. Plumbed inline, the seepage flows from the first tank into the second (Figure 3). Two PVC gate valves were installed, one before the tanks and the second in between the tanks. The first valve regulated the flow into the tanks and provided access for turning off flow for



maintenance. The second valve was used to stop flow into the second tank for sampling. The first tank was sampled through a T-fitting installed just before the second gate valve. The second tank was sampled from the effluent end of the bioreactor. The tanks are set on concrete pads and secured by two metal straps. Four inch (10.2 cm) PVC pipe was used for all plumbing. Once in place, the reactors were internally plumbed. The tanks came with an influent and effluent pipe about 12 inches (30.5 cm) in length which curved 90° towards the bottom of the tank. The influent piece was extended to the bottom of the tank using a 4 inch (10.2 cm) piece of schedule 40 PVC pipe approximately 5 feet (1.5 m) in length. A right angle connector then connected the influent pipe to a perforated pipe that runs the bottom length of the tank. Plumbing the tanks this way promotes up flow percolation. A sediment sock was placed over the bottom pipe to keep sediment from settling in the pipe. This sock was later removed due to Fe precipitate covering and clogging the sock.



Figure 4. Picture of the bioreactors installed at Guy Cove. The picture shows the progression of the seepage through the bioreactors and to the wetland.

The treatment matrix, which filled about two-thirds of the tank was a mixture of compost, hardwood mulch, and creek sediment. Compost was obtained from the University of Kentucky's Animal Research Center located in Woodford County Kentucky. Hardwood

mulch was purchased from a local contractor, Red River Ranch LLC. Creek sediment was collected from a routine cleaning of a weir in the Clemons Fork stream located in Robinson Forest. Compost, mulch, and creek sediment were mixed with a backhoe. An excavator was used to transport the mixed material to the reactor where it was manually shoveled into the bioreactors. There are two manholes on each end of the tanks (influent and effluent) for access. Within each manhole, a silver/silver chloride Mettler Toledo InLab redox probe was installed (two probes per tank) and sealed with a cover. A wetland was built on the downstream side of the bioreactors for further treatment (Figure 4). The wetland was created through the installation of an embankment to flood the area. Approximately 2 feet (61 cm) of hardwood mulch was placed as the uppermost layer of the wetland. Draining the wetland is a 36 inch (91 cm) black corrugated drain pipe. The wetland treats both the effluent from the bioreactors and also excess seepage from the underdrain. Effluent from the wetland flows into a constructed channel which discharges to the original Guy Cove stream.

### Sample Collection and Analysis

Water samples were collected every two weeks from December 2008 to October 2009 from the influent, the first tank, the second tank, and the effluent of the wetland sampling locations. Two 250mL Nalgene® water bottles were filled at each sampling location and immediately placed in a cooler for transport to the laboratory. Water temperature (°C), electrical conductivity ( $\mu\text{S cm}^{-1}$ ), dissolved oxygen ( $\text{mg L}^{-1}$ ), pH, and Eh (mV) were measured in the field, using Yellow Springs Instrument (YSI) 610D and 556 MPS environmental monitors. Oxidation Reduction potential was measured in the tanks using a Fisher Scientific Accumet AP62 pH/mV meter with a silver/silver chloride Mettler Toledo InLab Redox Probe. The Eh values were corrected to reflect a standard hydrogen electrode.

At the laboratory, one sample from each sampling point was acidified ( $\text{pH} < 2.0$ ) using nitric acid. A portion of the acidified sample was placed in a 50 mL centrifuge tube and used for the analysis of Fe and Mn. All samples were stored in a refrigerator until analyzed. Water samples were analyzed in the lab for pH, alkalinity, EC,  $\text{SO}_4$ , Fe, Mn, Cl, Ca, Mg, Na, K,  $\text{NO}_3$ , and  $\text{NH}_4$ . EC was analyzed using a YSI 35 conductance meter. Anionic solutes,  $\text{SO}_4$ , Cl, were analyzed on a Dionex ion chromatograph system 2500 (ISC 2500). The set-up contained an AS50 auto sampler, IS25 chromatograph, LC25 chromato-oven, and an EG50 eluent generator. Nitrogen containing compounds,  $\text{NO}_3$  and  $\text{NH}_4$ , were analyzed with a Braun:Luebbe Auto Analyzer 3 using the Colorimetric procedure. Alkalinity and pH were analyzed with an Orion 940/960 auto-titration combo. Samples were titrated to a 4.6 endpoint with 0.02 N HCl. Cationic (Ca, Mg, Na, Mn, and K) solutes were analyzed with a GBC SDS-276 atomic absorption spectrophotometer using the Direct Air-Acetylene Flame Method. Iron was analyzed on a Solaar M5 ThermoElemental atomic absorption spectrometer.

### Sequential Extraction

Substrate samples were collected from the bioreactor tanks in October 2009 and sequentially extracted following the procedure of Maharaj et al. (2006). The purpose of the sequential extraction was to evaluate mechanisms for removal of Fe and Mn within the passive treatment system. A one gallon zip lock bag was filled with substrate from two locations in each tank and three points in the wetland. Bio1-1 was collected from the influent side of the first tank and Bio1-2 was collected from the effluent side of the first tank. The

second tank was sampled in a similar fashion as the first tank and labeled Bio2-1 and 2-2, respectively. Wetland samples were collected at the influent (wetland up), middle (wetland mid) and effluent (wetland down) locations. The samples were transported to the University of Kentucky in a cooler and placed in a refrigerator for storage. A sub-sample from each bag was dried in an oven at 50°C for one week. Once dried, the samples were ground to a uniform size (<2mm) using a Humboldt Testing Equipment mechanical grinder. A 1g sample was used for the extraction and the procedure was replicated ( $n = 2$ ) for each sample. Table 2.2 provides a summary of the extraction procedure.

### Statistical Analysis

Statistical analyses were conducted using Sigmaplot 11 (Systat software 2008). Repeated measures ANOVAs using the Holms Sidak method were used to compare the significance of multiple groups of data of unequal variance, with student t-tests using the Mann Whitney U statistic for the comparison of two groups of data.

## Results

Mean water quality parameters for the passive treatment system are presented in Table 1. Statistical comparisons between the influent and other sampling points are summarized in Table 2. A separate test was conducted to compare the two tanks. The amount of seepage flowing from the underdrain was greater than the bioreactors could treat and excess seepage was diverted around the bioreactors. As such, the wetland was treating both seepage from the underdrain and effluent from the reactors. Because of this, the bioreactors were not compared directly to the wetland. Comparisons for oxidation reduction potential among tanks are listed in Table 3.

### Oxidation Reduction Potential

There were two sampling points in each bioreactor (1 and 2) for sampling Eh. The mean Eh levels for the six sample locations were 356 mV (influent), 113 mV (Bio1-1), 127 (Bio1-2), -23 mV (Bio2-1), -16 mV (Bio2-2), and 197 mV (effluent). Statistical differences among sample locations are summarized in Table 3. The influent is significantly higher than the other sampling points. Both influent and effluent within the tanks are statistically similar; however Bioreactor 2 is significantly lower than Bioreactor 1. Reductions in Mn, Fe and SO<sub>4</sub> redox state (Mn<sup>4+</sup> to Mn<sup>2+</sup>, Fe<sup>3+</sup> to Fe<sup>2+</sup>, and SO<sub>4</sub><sup>2-</sup> to S<sup>2-</sup>) are associated with Eh values of 200 to 100 mV, -100 to 100 mV, and -200 to -100 mV respectively (Thompson 2009, Reddy et al. 2000). In general, the first tank obtained reducing conditions for Mn, but not SO<sub>4</sub> and Fe. Within the second tank, reducing conditions for both Mn and Fe were obtained. In the wetland, reducing conditions were observed for only Mn. It is important to note that the Eh value used was calculated as a mean for the entire data set starting (after restoration) December 2008 to October 2009. While temperature is not directly related to Eh, it is directly related to microbial activity. It has been shown that microbial activity doubles with each 10°C increase in temperature (Benner 2002, Hao 1996, Stumm and Morgan 1996, Widdel 1988) which effectively lowers the Eh. Over the summer/fall months (June – October) the mean Eh values for Bio1-1, 1-2, 2-1, and 2-2 were 85 mV, 9 mV, -115 mV, and 26 mV respectively. Fe and Mn reducing conditions were reached in both tanks along with SO<sub>4</sub> reducing

conditions from the 2-1 location. It is likely that 2-2 was much higher than 2-1 because the effluent pipe from 2-2 allowed for the passage of oxygen into the tank, effectively raising the Eh. Although average Eh values only indicated that sulfate reducing conditions occurred periodically, minimum values recorded for each bioreactor locations were -163 mV (1-1), -183 mV (1-2), -271 mV (2-1), and -408 mV (2-2). Considering that the redox probes were installed near the top of the bioreactors in the water column, reducing conditions within the substrate are unknown. We speculate that Eh was much lower within the substrate and that sulfate reduction was achieved in that zone.

### Temperature and Dissolved Oxygen

The range of temperature for the passive treatment system was 12.7 °C for bioreactor 1 to 14.6 °C for the effluent. No point of the treatment system was statistically different than the influent. Dissolved oxygen means ranged from the influent at 5.4 mg/L to the effluent at 9.2 mg/L. The effluent DO was significantly greater than the influent DO (Table 2). Water sampled from the wetland was on the downstream side of a stand pipe and 24 feet of corrugated drain pipe. It is likely that the water was aerated within the drain pipe making DO or Eh higher on the downstream side of the berm.

**Table 1: Mean (standard error)Water Quality Parameters tested for the four sampling locations within the passive treatment system**

Parameter	Influent	Bio 1	Bio 2	Wetland Effluent
Temperature (°C)	13.8(0.55)	12.7(1.44)	13.5(1.48)	14.7(1.23)
DO (mg L <sup>-1</sup> )	5.4(1.17)	7.1(1.72)	6.6(1.56)	9.2(1.44)
Redox (mv)	363(124.2)	120 / 133 (41.2)/(42.3)	1.70454545 (39.4)/(42.3)	204(14.8)
pH	5.5(0.07)	6.1(0.09)	6.4(0.19)	6.5(0.09)
EC (uS cm <sup>-1</sup> )	1972(29.9)	2033(147.6)	2255(181.7)	1728(140)
Sulfate (mg L <sup>-1</sup> )	1543(56.4)	1480(61.8)	1486(100.2)	1371(79.5)
Iron (mg L <sup>-1</sup> )	3.7(0.68)	2.0(0.55)	1.0(0.29)	1.3(0.19)
Manganese (mg L <sup>-1</sup> )	10.1(0.82)	9.2(0.68)	9.6(0.62)	8.9(0.65)
Magnesium (mg L <sup>-1</sup> )	14.3(0.49)	14.7(0.49)	14.1(0.49)	14.0(0.48)
Calcium (mg L <sup>-1</sup> )	26.9(0.93)	26.6(1.02)	27.1(0.91)	26.8(0.94)
Potassium (mg L <sup>-1</sup> )	6.8(0.14)	7.1(0.12)	9.9(0.72)	6.8(0.22)
Sodium (mg L <sup>-1</sup> )	8.4(0.25)	8.3(0.21)	10.8(0.75)	7.9(0.29)
Alkalinity (mg L <sup>-1</sup> )	121.8(9.9)	420.3(46.2)	644.7(62.4)	315.2(21.9)
Nitrate (mg L <sup>-1</sup> )	0.04(0.01)	0.04(0.01)	0.13(0.05)	0.04(0.01)
Ammonium (mg L <sup>-1</sup> )	0.26(0.04)	0.14(0.02)	1.60(0.49)	0.18(0.33)
Chloride (mg L <sup>-1</sup> )	2.4(0.19)	36.7(14.6)	74.7(16.5)	2.3(0.19)

**Table 2: P-values from t-tests conducted between sampling points within the passive treatment system. Bold values are significant ( $\alpha = 0.05$ )**

Parameter	In† vs. B1	In vs. B2	In vs. Eff	B1 vs. B2
Temperature (°C)	0.904	0.847	0.658	0.585
DO (mg L <sup>-1</sup> )	0.385	0.697	<0.001	0.83
Redox (mv)	0.07	0.058	<0.001	0.934
pH	<0.001	<0.001	<0.001	<b>0.005</b>
EC (uS cm <sup>-1</sup> )	0.909	0.781	0.194	0.799
Sulfate (mg L <sup>-1</sup> )	0.266	0.371	0.128	0.936
Iron (mg L <sup>-1</sup> )	0.102	<b>0.008</b>	0.052	0.144
Manganese (mg L <sup>-1</sup> )	0.671	0.972	0.261	0.617
Magnesium (mg L <sup>-1</sup> )	0.694	0.055	0.455	0.214
Calcium (mg L <sup>-1</sup> )	<b>0.004</b>	0.5687	<b>0.021</b>	<b>0.028</b>
Potassium (mg L <sup>-1</sup> )	0.694	0.568	0.145	0.308
Sodium (mg L <sup>-1</sup> )	0.509	<0.001	0.736	<b>0.002</b>
Alkalinity (mg L <sup>-1</sup> )	0.886	0.993	0.959	0.934
Nitrate (mg L <sup>-1</sup> )	0.688	0.795	0.5	0.639
Ammonium (mg L <sup>-1</sup> )	0.558	0.522	0.387	0.558
Chloride (mg L <sup>-1</sup> )	0.904	0.847	0.658	0.585

†In = influent; Eff = effluent.

**Table 3: Oxidation reduction potential p-values for sampling points within the passive treatment system, points include the bioreactors and the effluent from the wetland. A one-way repeated measures ANOVA was conducted to compare the Influent, Bio 1-1, 1-2, 2-1, and 2-2. A t-test was used to compare influent to effluent**

	Bio 1-1	Bio 1-2	Bio 2-1	Bio 2-2	Effluent
Influent	<0.001	<0.001	<0.001	<0.001	<0.001
Bio 1-1		0.784	0.008	0.012	
Bio 1-2			0.004	0.006	
Bio 2-1				0.89	

### pH and Alkalinity

Average pH for the treatment ranged from 6.2 (influent) to 6.8 (effluent). The effluent was significantly higher than the influent. Alkalinity means ranged from the influent at 122 mg L<sup>-1</sup> to Bioreactor 2 at 654 mg L<sup>-1</sup>. Both bioreactors were statistically different and significantly higher than that of the influent (Table 2). The effluent was also statistically higher than the influent. The treatment system was designed to raise the pH of the seepage



and did so gradually through each portion of the treatment system. The wetland however was the most successful element of the treatment system at raising the pH. Alkalinity was dramatically increased inside the bioreactors due to the substrate used in the reactive matrix.

### Electrical Conductivity and Sulfate

No statistical differences were observed for EC with means ranging from 2,003 to 2,252  $\mu\text{S cm}^{-1}$ . Sulfate means ranged from 1,580 to 1,404 mg/L for the influent to effluent respectively. Bioreactor 1, 2 and the effluent are all statistically similar to the influent (Table 2). As mentioned above, there was a lag time associated with the season in which the bioreactors were installed. Though no significant reduction in  $\text{SO}_4$  was observed, an approximate 6.6% (Bioreactor 2) and 11.1% (effluent) reduction of  $\text{SO}_4$  was measured over the sampling period. Reductions in  $\text{SO}_4$  during the summer/fall months (June through October) for Bioreactors 1 and 2 and the effluent were 10%, 21%, and 19%, respectively. Sulfate reductions for our system were similar to those described by Benner et al. (1997, 1999, 2002) over a three year period with a total of 30%  $\text{SO}_4$  removal. Dvorak (1992) and McCauley (2009) reported similar reductions of 17-20% and 18-27% from mesocosm scale bioreactors.

### Iron and Manganese

According to the overall mean Eh values, both Mn and Fe reducing conditions occurred over the sampling period. Fe ranged from an influent mean of 3.7 mg  $\text{L}^{-1}$  to bioreactor 2 mean of 0.96 mg  $\text{L}^{-1}$ . Iron reductions were only significant between the influent and bioreactor 2. Mn showed no statistical differences with means ranging from 9.5 to 8.3 mg  $\text{L}^{-1}$ . Fe reductions for each section of the treatment system bioreactor 1, 2, and effluent were 47%, 74%, and 64% for the sampling period and for the summer/fall months (June thru October) were 74%, 88%, and 78%, respectively. Mn reductions were 7%, 5%, and 12% for the sampling period and during the summer months 9%, 13%, and 18% for bioreactors 1, 2 and the effluent, respectively. Even though only bioreactor 2 reduced Fe significantly, observable reductions did occur for both Fe and Mn across the passive treatment system. Fe precipitation is likely due to pH and alkalinity concentrations from the dissolution of carbonates. Similar results were shown by Carrera et al. (2001) with the presence of a carbonate creating an optimal environment for Fe precipitation in which a 90% reduction was observed. Mn reductions are more than likely due to sorption or ion exchange with the organic substrate within the tanks.

### Nitrate and Ammonium

Nitrate means were statistically similar with a range of 0.04 to 0.13 mg/L. Ammonium ranged from 0.13 (bioreactor 1) to 0.91 (bioreactor 2). Bioreactor 1 and the effluent were significantly different from the influent. Bioreactor 1 was also significantly lower than bioreactor 2 (Table 2). We believe that  $\text{NO}_3$  and  $\text{NH}_4$  concentrations in the tanks were derived from the mulch and compost mixture. Bioreactor 2 had elevated levels of both  $\text{NO}_3$  and  $\text{NH}_4$  which may have been due to the mixture containing more compost. Stumm and Morgan (1996) state that excess ammonium is easily dissolvable in water. If oxidized,  $\text{NH}_4$  can become  $\text{NO}_3$ , and in reduced conditions  $\text{NO}_3$  can be converted to  $\text{NH}_4$ . Within the tanks it is possible that both reactions were taking place.

## Na, K, Ca, Mg and Cl

Sodium averages ranged from 7.9 – 9.7 mg L<sup>-1</sup> with no significant differences among sample locations. Potassium means ranged from 6.8 mg L<sup>-1</sup> (effluent) to 8.8 mg L<sup>-1</sup> (bioreactor 2). Bioreactor 2 is significantly higher than both the influent and bioreactor 1 (Table 2). Means ranged from 27.4- 27.6 mg L<sup>-1</sup> and 14.2- 14.7 mg L<sup>-1</sup> for Ca and Mg, respectively. No significant differences were observed across the sampling points for either Ca or Mg. The range of Cl values was from 2.3 to 50.5 mg L<sup>-1</sup>. There were no significant differences across the treatment sampling points. Chloride concentrations are likely from the compost used in the treatment matrix. Values for the first few sampling events were very high once the concentrations declined there were no significant differences among sample locations.

## Sequential Extraction

Figure 5 shows the speciation of Fe (a) and Mn (b) from substrate samples collected from the bioreactors. Iron (a) predominately was present as organically bound, poorly crystalline and residual fractions. Simple sorption accounts for the organically bound fraction. The poorly crystalline fraction comes from Fe precipitating out as an oxyhydroxide often referred to as “yellow boy”. During sulfate reduction, a Fe sulfide forms as the residual fraction. Conditions for precipitating iron were very favorable due to the pH being circum-neutral. Some water soluble Fe was present but very little accounted for Fe retention within the system. The exchangeable Fe fraction was undetected in the samples tested.

Manganese was present in all six fractions with the poorly crystalline and organically bound fractions higher in the bioreactors while the exchangeable phase was greatest in the wetland. The amount of easily dissolvable organic matter in the tanks created an optimal environment for Mn to sorb to organic matter. Sulfate reduction to a Mn sulfide was observed in each portion of the treatment process. A small portion of crystalline manganese was found in each sample and likely attributed the production of rhodocrosite (MnCO<sub>3</sub>) (Barton et al. 1999).

The preferable method of metal removal would have been to form a metal sulfide. Metal sulfides exist in the residual fraction and accounted for 8-41% of the Fe and 6-21% of the Mn. Neculita et al. (2008) reported 42-74% Fe and 30-77% Mn in the residual fraction in bioreactor. Likewise Edwards et al. (2009) reported less than 10% Mn in the residual in a mesocosm scale bioreactor. It is important to note that there was a wide array of removal mechanisms for Mn throughout the passive treatment system without one being overly dominant. Whereas removal mechanisms for Fe were dominated by three mechanisms: the organically bound, poorly crystalline, and residual fractions.

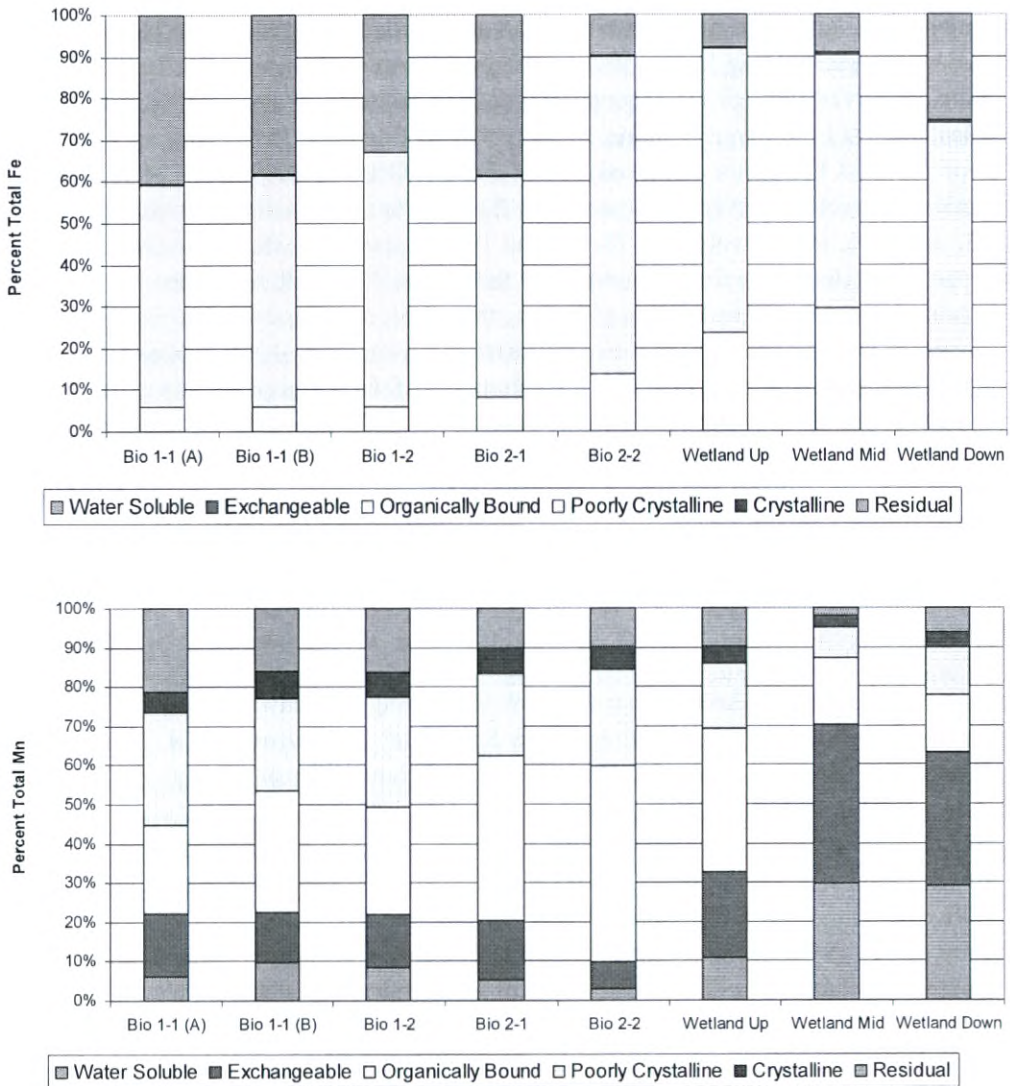


Figure 5. Speciation of iron (a) and manganese (b) from composite substrate samples collected in bioreactor and wetland substrates. Bio1-1 was sampled twice (A) and (B).

## Conclusions

Although pilot experiments using synthetic mine drainage showed promise for treating circumneutral mine drainage with elevated Mn and  $\text{SO}_4$  levels, success of the field deployed passive treatment system was limited at best. Because Fe levels were low in the Guy Cove mine drainage ( $< 4 \text{ mg L}^{-1}$ ) we chose not to include it in our synthetic drainage solution. The omission of Fe from the synthetic drainage was unfortunate as flow problems associated with Fe precipitation clogging the stand pipe and plumbing within the field deployed tanks persisted throughout the majority of the sampling period. The mixed chemistry of the mine drainage likely also contributed to the limited field success. Even with these problems, redox

conditions for Fe, Mn, and SO<sub>4</sub> reduction were attained for some periods of time. Sequential extraction of the substrate from the bioreactors and wetland suggested that both Fe and Mn sulfides were precipitating in the passive treatment system. Other mechanisms of removal were also observed through the sequential extraction including water soluble, exchangeable, organically bound, poorly crystalline, and crystalline fractions. Reductions in Fe, Mn, and SO<sub>4</sub> for the first bioreactor observed during June – October 2009 were 74, 9, and 10%, respectively. Levels observed in bioreactor 2 for the same parameters and time period were 88, 13, and 21%, respectively, and 78, 18, and 19%, respectively for the wetland. Treatment data spans only 10 months from January 2009 to October 2009. Although these problems may have caused the system to be less effective, there are ways to improve the system. One improvement would have been to move the reactors downstream of the wetland. This would have provided ample time for the Fe to precipitate out of the drainage and not cause problems in the tanks. Also the wetland would have begun to lower the Eh of the drainage which could be lowered further inside each one of the tanks. Another possible improvement to the passive treatment system would be to enlarge the wetland; however the wetland size was constrained by available area in the downstream location and is likely not foreseeable.

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